Rayleigh's Ratio for Benzene and the Problem of Absolute Light Scattering Determinations

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THE current controversy over the correct value for Rayleigh's ratio for benzene, or, in general, over absolute light scattering measurements, is bound to cause uncertainty in the many laboratories which use the light scattering method for determining molecular weights. Zimm[1] has already emphasized that the light scattering methods[2–4] which lead to the so-called "high" value for benzene have in their favor the fact that they produce correct molecular weight values. It must be assumed that Stamm and Button, who have made the most recent contribution to this controversy,[5] believe that this is due to an accidental compensation of errors. The purpose of the present communication is to point out that such an accidental compensation is most unlikely.

The light scattering literature is not rich in molecular weight determinations, which can be compared directly with reliable values obtained by other methods, on monodisperse molecules of a size suitable for accurate measurement. Perhaps the most extensive series is that of Halwer, Nutting and Brice[6] on the proteins β lactoglobulin, serum albumin, lysozyme, and ovalbumin. Using a light scattering method[3] which leads to a "high" value of 48.4×10^{-6} for Rayleigh's ratio for benzene at 436 mμ, they obtained molecular weights that differ by not more than 5 percent from the averages of modern determinations by other methods, notably, osmotic pressure, sedimentation and diffusion, x-ray diffraction, and amino acid analysis.

From Stamm and Button's Table I, the ratio of scattering by benzene to the excess scattering by the "Styron standard" (solution minus solvent) can be calculated as $27.59/122.1=0.226$ for 436.57 mμ and $10.37/45.42=0.228$ for 546.07 mμ. Our ratios for these quantities are $48.4/209=0.232$ for 436 mμ (3) and $17.6/78.2=0.225$ for 546 mμ. Their *relative* scatterings are therefore in close agreement with ours and with those of Carr and Zimm. However, their *absolute* value for Rayleigh's ratio for benzene is 0.570 times our value, and the excess scattering of the Styron standard 0.584 times our value for 436 mμ. Thus, if in our measurements on the proteins our turbidity values were replaced by theirs, the calculated molecular weights would be about 42 percent *lower* than accepted values determined by independent methods. Clearly, Stamm and Button must believe that one or more of the other measurements that are involved in our determinations must bear a compensatory error of 42 percent.

Our results were obtained using the Debye light scattering equation, $Hc/\tau=1/M+2Bc$, where M is the molecular weight and τ the turbidity. The protein concentration, c, was determined by standard methods, i.e., either the pure, air-dry protein (containing a few percent moisture) was weighed out directly, or an aliquot of solution was dried to constant weight at 100°. It is not conceivable that c could be in error by the amount required. The constant H involves the wavelength of the incident light, the refractive index of the solvent, and the specific refractive increment, $(n-n_0)/c$, where n and n_0 are the refractive indices of the solution and solvent, respectively. The only one of these quantities about which there can be any doubt is the specific refractive increment. However, our values[6] agree within 2 percent with other independent values.[7–10] As for the slope term, $2Bc$, the slopes of the Hc/τ *vs* c plots are so small that a maximum error of 11 percent would be made by neglecting the slope term entirely. Nor can inadequate clarification of solutions or the presence of fluorescence be invoked, since these would make the results too high, whereas we are seeking a supposed error that makes them too low.[11]

The evidence leads us to conclude that a systematic error exists in the method of Stamm and Button for the determination of absolute light scattering power. If their absolute values are correct, they must show that these values lead to correct molecular weights; that our molecular weight results and those of Carr and Zimm and others are correct only through compensation of errors; that the agreement between turbidities determined by transmittance and by 90° scattering obtained by several investigators[2,4,12] is fortuitous; or that Debye's relationship for determining molecular weights by light scattering is not valid.

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[11] In support of our values are the recent results of Rhees and Foster, Iowa State Coll. J. Sci. **27**, 1 (1952), who obtained values close to ours for serum albumin, ovalbumin and lysozyme, using an independent light scattering method. Their instrument was calibrated by the transmission method, using Ludox colloidal silica. Mommaerts[12] has shown that this method leads to turbidity values in agreement with our own. Edsall, Edelhoch, Lontie, and Morrison, J. Am. Chem. Soc. **72**, 4641 (1950) obtained a value for serum albumin in good agreement with ours, using our value for the Styron standard to calibrate their instrument.
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